

**Abbreviated Identification of
Bacteria and Yeast**

NCCLS Guideline M 35 A
Mary K. York, Ph.D. ABMM

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References Prove Value

- Doern, G., R. Vautour, M. Gaudet, and B. Levy. 1994. Clinical impact of rapid *in vitro* susceptibility testing and bacterial identification.
 - ◆ MIC 9.6 h from colonies vs. 25.9 h- showed less mortality, length of stay and orders of laboratory tests
- Barenfanger, J., C. Drake, and G. Kacich. 1999. Clinical and financial benefits of rapid bacterial identification and antimicrobial susceptibility testing.
 - ◆ evaluated evening vs. next day - 5 h difference - length of stay and cost was significantly less

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Pros and Cons of Rapid Methods

- Pros
 - ◆ Less work than standard methods
 - ◆ Results are out faster
 - ◆ Less cost
- Cons
 - ◆ Requires technical expertise for accuracy
 - ◆ Cannot be applied to all cases
 - ◆ May disrupt workflow

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Criteria Used by Committee for Rapid Tests

- Only for specific organisms
- Errors must not have a negative impact on patient care
- Accuracy must be > 95%
- Emphasis on organisms that have unique reactions
- Results are not presumptive if all criteria met-
cpt 4 code issue

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Factors to Keep in Mind

- Not for direct specimens
- All conditions must be met
- Keep isolate for future testing if needed

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Technologist must....

- Begin with pure colony
- Recognize what it could be from typical colony morphology
- Perform rapid tests accurately and read them correctly
- Often do Gram stain

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Supervisor must.....

- Validate the competency of the staff doing tests
- Check to see that all tests are done
- Be sure that procedures are written and QC is done at appropriate intervals

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CLIA '88

- *All quality control activities must be documented.*
- *The laboratory must check positive and negative reactivity with control organisms*
- *Each new lot/shipment of reagents, commercial tests, or biochemical test media prior to being used on patient specimens.*

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CLIA '88

→ and

- (1) Each day of use for catalase, coagulase, beta-lactamase, and oxidase reagents and DNA probes;
 - (2) Each week of use for Gram stain, bacitracin, optochin, ONPG, X and V discs or strips; and
 - (3) Each month of use for antisera...
- Does not address ID disks, rapid indole, Staph Latex reagents, etc.....*

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CLIA 2003

→ All quality control activities must be documented

◆ The laboratory must check positive and negative reactivity with control organisms

- (1) Each day of use for DNA probes and beta-lactamase (ex. cefinase);
- (2) Each week of use for Gram stain and AFB stains; and
- (3) Every 6 months of use for antisera...
- (4) Each new lot/shipment of reagents, commercial tests, or biochemical test media prior to being used on patient specimens.

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Organisms covered by NCCLS

Gram Negative Bacilli

- *Escherichia coli*
- *Haemophilus influenza*
- *Moraxella catarrhalis*
- *Proteus mirabilis/penneri*
- *Proteus vulgaris*
- *Pseudomonas aeruginosa*

Gram Positive Cocci

- *Enterococcus* species
- *Staphylococcus aureus*
- *Streptococcus agalactiae*
- *Streptococcus pneumoniae*
- *Streptococcus pyogenes*

Yeast

- *Candida albicans*
- *Candida glabrata*
- *Cryptococcus neoformans*

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Organisms covered by NCCLS

Anaerobic Gram Negative Bacilli

- *Bacteroides fragilis* group
- *Bacteroides urealyticus*
- *Bilophila wadsworthii*
- *Prevotella* species
- *Prevotella intermedia*
- *Porphyromonas* species
- *Fusobacterium nucleatum*

Anaerobic Gram Positive Bacilli

- *Clostridium difficile*
- *Clostridium perfringens*
- *Clostridium septicum*
- *Clostridium sordellii*
- *Clostridium tetani*
- *Propionibacterium acnes*

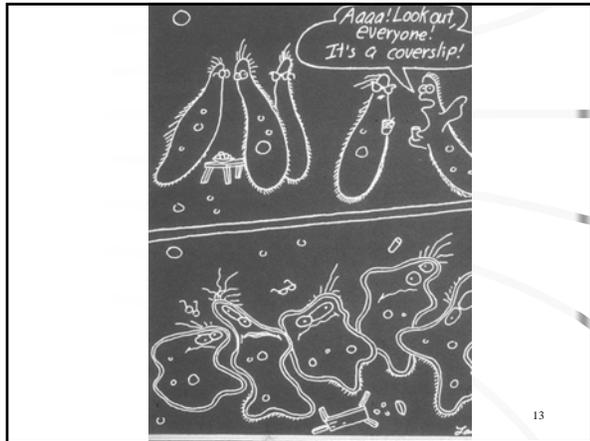
Anaerobic Gram Negative Cocci

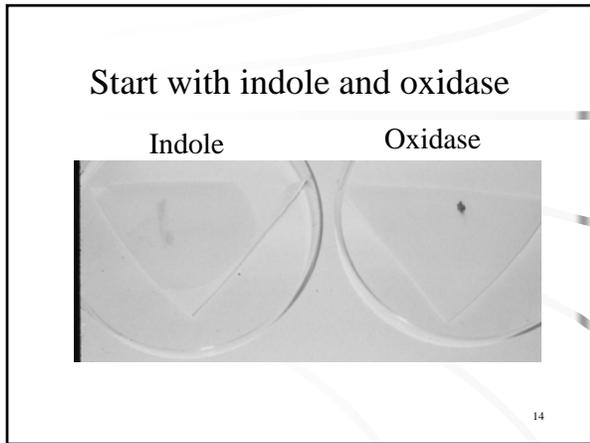
- *Veillonella* species

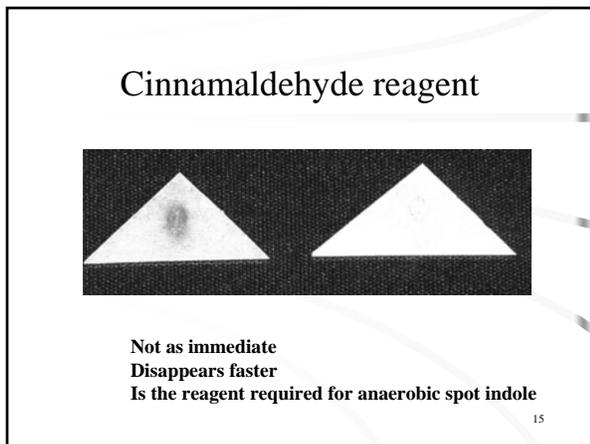
Anaerobic Gram Positive Cocci

- *Peptostreptococcus* species

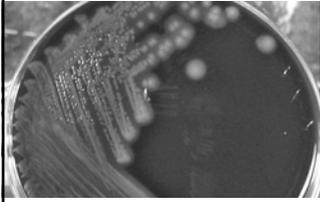
12







Identification of *E. coli*



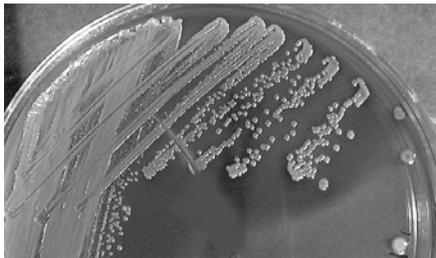
- Indole +
- Oxidase -
- Gram-negative rod
- Beta-hemolytic

= *E. coli*

Limitation: some *Proteus* and *Morganella* and all *Edwardsiella* are hemolytic. These species are lactose-negative.

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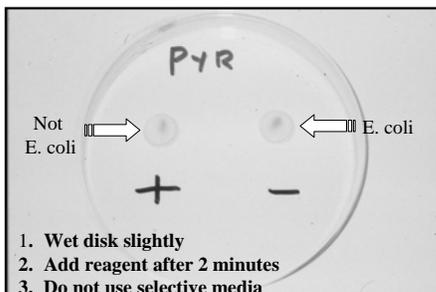
What if it is not hemolytic?



Lactose +
and

17

PYR negative = *E. coli*



1. Wet disk slightly
2. Add reagent after 2 minutes
3. Do not use selective media

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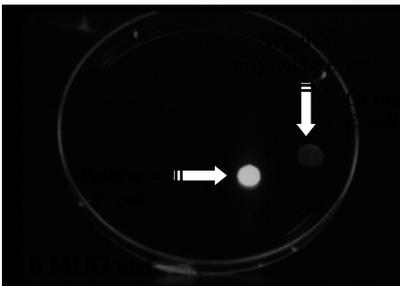
PYR reactions of GNR

| | |
|--------|---|
| Some + | V |
| + | V |
| Some + | + |
| Some + | - |
| | + |
| | - |
| | + |



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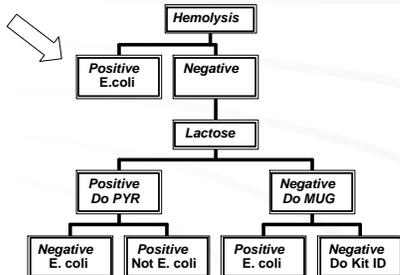
What if it is not lactose-positive?



y be
O157

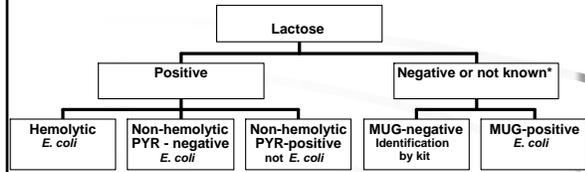
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CLSI Algorithm for E. coli: Indole +; Oxidase -



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Algorithm for some labs... Indole + and oxidase -



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Limitations

- Beta hemolytic, lactose-negative should be limited to UTI and geographic areas that lack hemolytic *Morganella* and *Proteus*.
- Take colony from BAP that corresponds to MAC or EMB
- Do not use MUG for GI specimens, except to identify *E. coli* O157.

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Accuracy of 1064 Indole + oxidase- strains = 99.7%

- 294 were hemolytic and *E. coli*.
- 628 were lactose positive and PYR negative and *E. coli*.
- 65 were MUG positive and *E. coli*.
- 13 were MUG negative and needed kit to identify as *E. coli*.
- 64 were not *E. coli* but 3 were called *E. coli*

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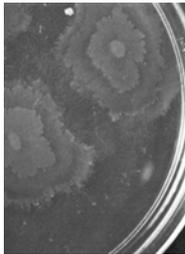
Cost savings

- \$3100 in reagents - 987 kits omitted
- 70 hours of technologist time

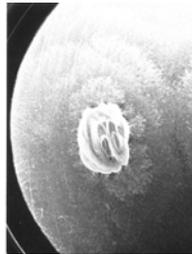
York, M.K., E.J. Baron, M. Weinstein, R Thomson, and J.E. Clarridge. 2000. A Multi-Laboratory validation of rapid spot tests for identification of *Escherichia coli*. J Clin Microbiol 38: 3394-3398.

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Spreading *Proteus*



MacConkey



BAP

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Proteus identification= Spreading colony plus....

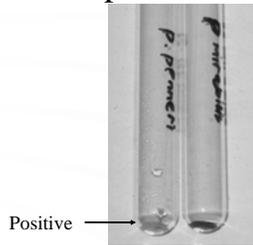
- ♦ Indole positive: → *Proteus vulgaris*
- ♦ Indole negative: → Ampicillin susceptible:
Proteus mirabilis
→ Ampicillin resistant
→ Maltose-negative or Ornithine-positive: *P. mirabilis*
→ Maltose-positive or Ornithine-negative: *P. penneri*

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Proteus species

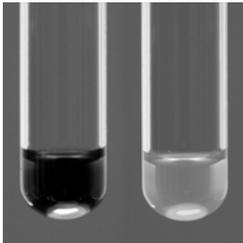
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Rapid maltose



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Rapid Urea



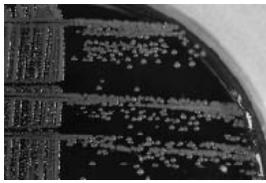
- Make suspension of organism
- Add disk
- Incubate 2 h
- Read urea
- Add HCl- ~~not~~ nec.
- Add FeCl_3

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Other Rapid Urea Positive species

- *Brucella*
- *Helicobacter pylori*
- *Bordetella* species
- Some *Corynebacterium*

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- Grows on BAP
- Coccobacillus
- Catalase- positive
- Oxidase- positive
- Urease- positive

- This is *Brucella* unless proven otherwise
- Work in a BSC
- Confirm with serology
- Reportable disease
- Bioterrorism



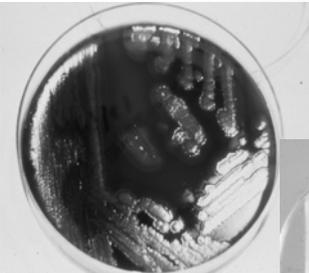
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Brucella Diagnosis

- Symptoms are non-specific
- Onset is insidious
- Risks are eating raw dairy products or working in a microbiology laboratory
- Without diagnosis there is no appropriate treatment:
Doxycycline plus rifampin

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Pseudomonas aeruginosa



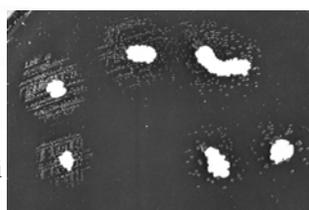
- Oxidase +
- Indole -
- Metalic or mucoid
- Fruity odor

CF patients:
Confirm with
colistin or
poly B disk

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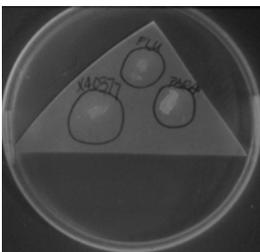
Identification of *Haemophilus influenzae*

- Small gram-negative coccobacilli
- Growth of large colonies only on CHOC in 24 h or around staphylococci
- And.....



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ALA test negative - 2 h 35°C read under UV light

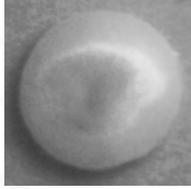


1. *Francisella* grows on CHOC in 48 h but is small colony
2. Cannot differentiate *H. influenzae* from *H. haemolyticus*; the latter is hemolytic on horse or rabbit blood agar.

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•You can either make the reagent or buy it

•Recipe in NCCLS M35-A and handout



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Haemophilus species

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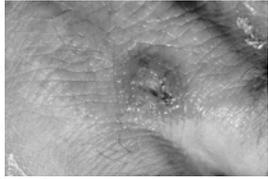
Horse Blood Agar



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Case Study

- ◆ 36 y/o male with HIV
- ◆ Camped in Yosemite
- ◆ Non-healing, erythematous 3 mm cyst on neck
- ◆ FNA aspirate and biopsy
- ◆ Gram stain negative
- ◆ GNR grew on chocolate in 3 days
- ◆ Patient treated with ciprofloxacin & did well



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Day 3- GNR growing on Choc
catalase +; urease- ;oxidase-
*Vitek NHI = 99% Actinobacillus
actinomycetemcomitans*

Day 4- Satellite negative

ALA neg; motility- ;NH 2520 No ID

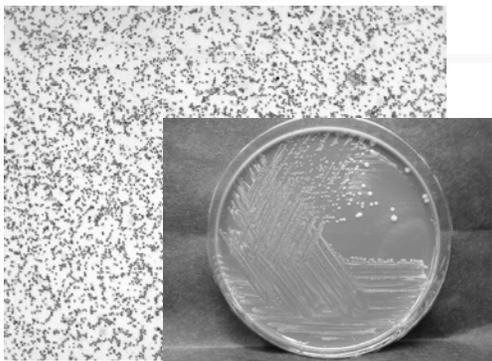
Day 5- Tech sets up MIC- no growth day 7

Day 10- KB set up

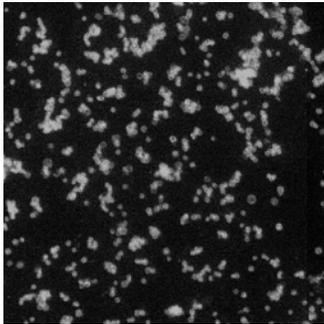
Day 12- KB penicillin R

Day 40- MDL reports ID done by FA

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Francisella tularensis - BSL III

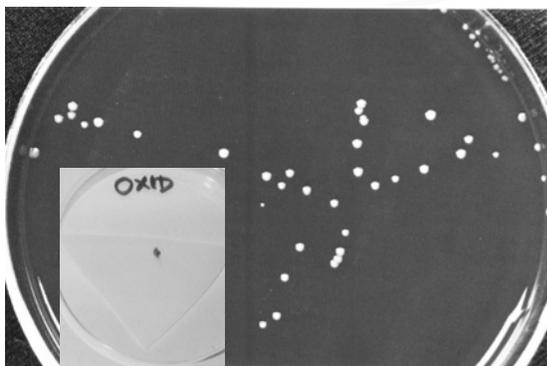


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Francisella tularensis

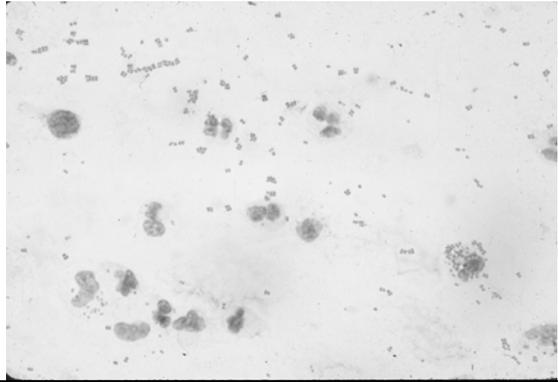
- ◆ If growth takes longer than 24 h, may be *Francisella* which will not satellite,
 - ALA-negative.
 - Grows on CHOC but not on BAP.
 - Oxidase-negative; catalase weak.
 - No kit will identify
 - Do cefinase-result is positive.
 - Send to health department if cefinase-positive
- ◆ Rabbit fever, tick, mosquito & fly bites
- ◆ Bioterrorism

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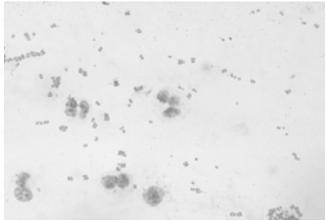
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Moraxella catarrhalis



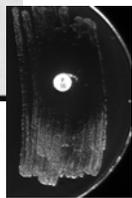
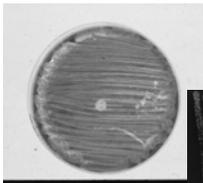
Confirmatory Identification
of *M. catarrhalis*

- Gram negative diplococci
- Grows on BAP
- Oxidase positive
- Butyrate (or indoxyl acetate) positive in 5 min



Actually all *Moraxella* are butyrate + but the others are coccobacilli

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- ◆ Inoculate plate and put Pen disk on it
- ◆ Perform Gram stain from around the disk
- ◆ Do on subs of positive blood cultures- polymyxin B and vancomycin too!

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→ May use indoxyl acetate
 → Same method and color
 → Can be used for identification of *Campylobacter jejuni*
 (*C. lari* is negative)

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Campylobacter jejuni

- ◆ Requires microaerobic environment
- ◆ Oxidase - positive
- ◆ Catalase - positive
- ◆ Curved rod
 - Hippurate - positive
 - If hippurate - negative; indoxyl acetate - positive and cefazolin R identifies
 - Nal Acid or cipro R & cefazolin R & indoxyl acetate -negative is *C. lari*



Rapid Hippurate

- Inoculate broth
- Incubate 2 h
- Add ninhydrin
- Observe blue color



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In Summary for Gram negative rods

- If growing on MAC, do
 - ◆ Indole
 - ◆ Oxidase
 - ◆ Kit if not *E. coli*, *Proteus* or *Pseudomonas*
- If not growing on MAC, do
 - ◆ Catalase
 - ◆ Oxidase
 - ◆ Gram stain
 - ◆ Generally kits are not helpful here

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Staphylococcus aureus



Latex agglutination

Identification of *S. aureus*

- Grows as opaque, white colony
- May be beta hemolytic
- Catalase-positive
- Gram-positive cocci in clusters
- Slide, tube or agglutination positive

Rapid tube coagulase is 4 h at 35°C; then must go to 25°C for 20 h more if negative

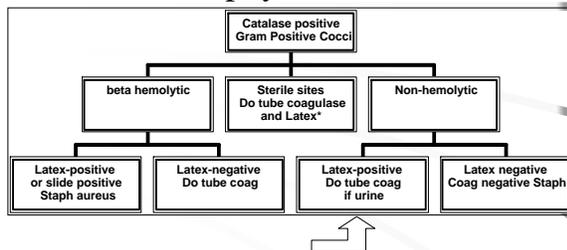
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Limitations

- Rapid Tube Coag is 4 h at 35°C; then must go to 25°C for 20 h more if negative
- *S. lugdunensis* and *S. schleiferi* can be slide and Latex positive
- *S. saprophyticus* (and rare others) can be Latex positive
- MRSA can be Latex negative

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Algorithm for Latex testing for Staphylococci



Hemolysis is defined as present in 18 h - not 48 h; not under colony

*Do PYR if tube coagulase negative to rule out *S. lugdunensis*⁵⁶

Case study

- Positive blood culture from patient with endocarditis
- Slide or Latex coagulase positive
- Tube coagulase negative
- PYR positive
- Ornithine positive

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Staphylococcus lugdunensis

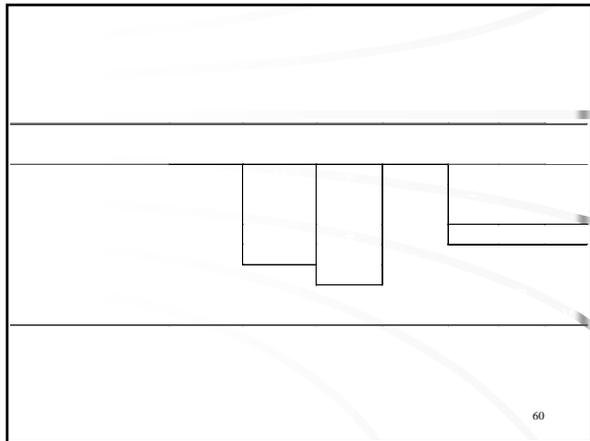
- Identification important to treatment
- CLSI now uses *S. aureus* methicillin breakpoints for this species



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Use PBP2a on CoNS with MICs of less than 4 µg/ml





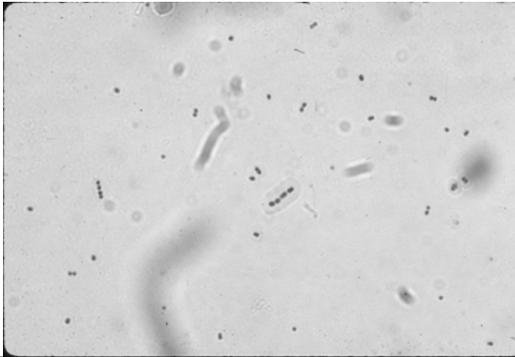
60

Streptococcus pneumoniae



30 min at 35°C 61

Quellung reaction



S. pneumoniae Identification

- Colonies small, transparent- may be mucoid
- Gram positive cocci - lancet shaped in pairs
- Catalase negative
- Bile soluble

Limitation: Not all are bile soluble but all bile soluble are *S. pneumoniae*

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Enterococcus Identification

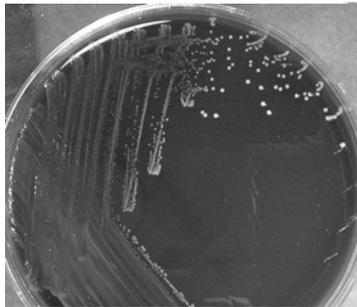
- Large (1mm) non hemolytic colony
- Gram positive cocci in pairs and chains
- Catalase negative
- PYR +



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Case Study

- Positive blood culture post-partum
- PYR positive
- Laboratory called it *Enterococcus*
- Gram stain alerted to misidentification
- Called contaminant: patient discharged.



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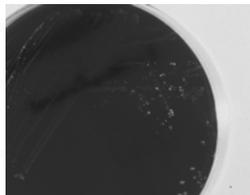
Other PYR-positive Cocci

Aerococcus - tetrads

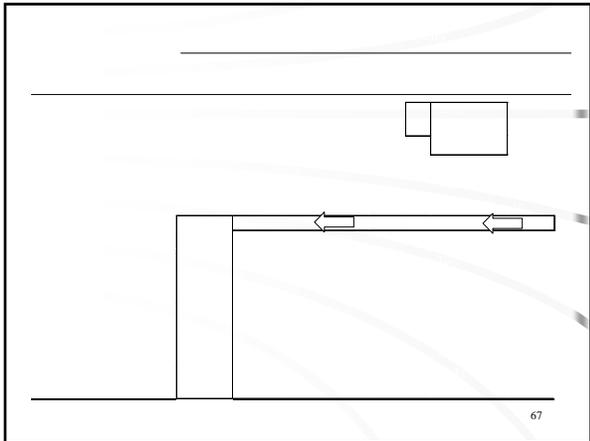
Vagococcus - motile

Vancomycin resistant is always *Enterococcus* if PYR +

Cannot separate *Enterococcus* from *Lactococcus*



66



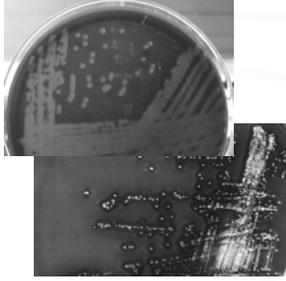
Identification of *S. pyogenes*

- Colonies: dry, peaked >0.5 mm in diameter
- Beta hemolytic
- Catalase-negative
- Gram-positive cocci in pairs and chains
- PYR-positive or positive particle agglutination

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***Enterococcus* can be beta**

Enterococcus look different

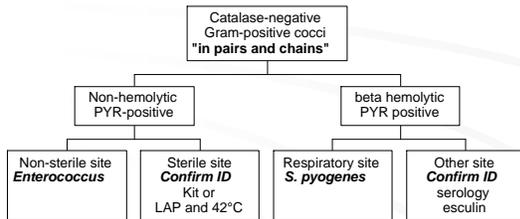


- *Enterococcus* colonies are larger with less defined β zone
- Do rapid esculin if there is a concern
- *Enterococci* are esculin positive

Group A Strep

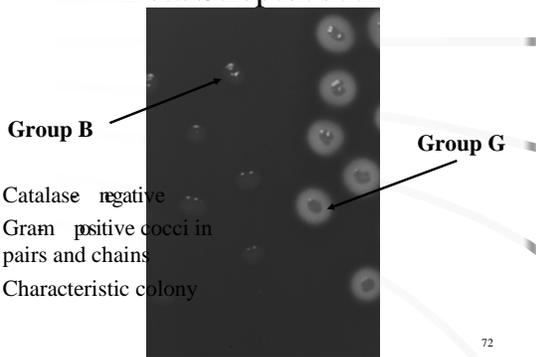
70

PYR - Positive Algorithm



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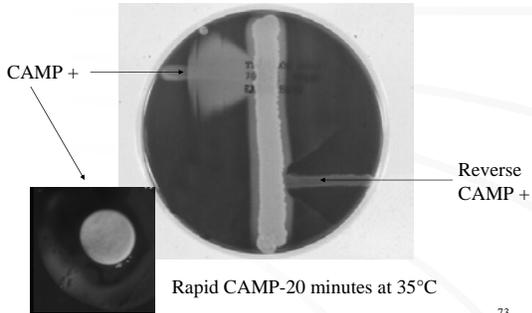
Beta Streptococci



- Catalase negative
- Gram positive cocci in pairs and chains
- Characteristic colony

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and CAMP test positive



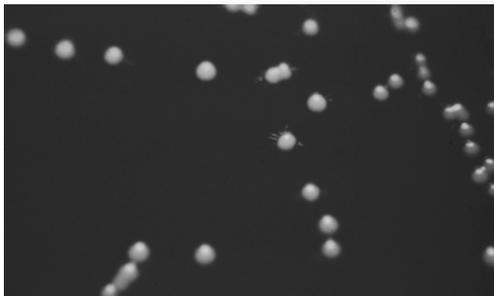
...or hippurate positive

Limitation: Do not do hippurate on non-hemolytic colonies- Other Streptococcus can be positive



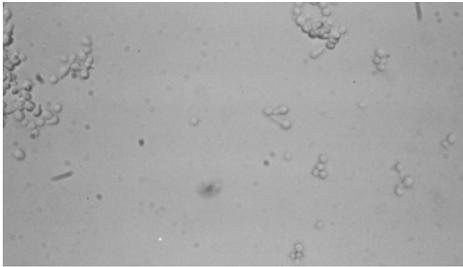
74

Candida albicans



75

Candida albicans



Only use 100% fetal or newborn calf serum-2 h 35°C

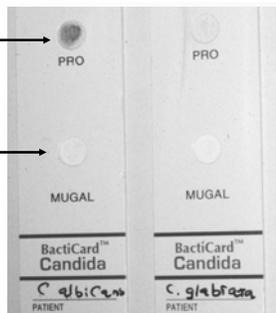
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Candida albicans = Positive for both enzymes

Add developer
at 5 min

Read under
UV light
Like MUG

Use antibiotic
free media



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Candida albicans Limitations

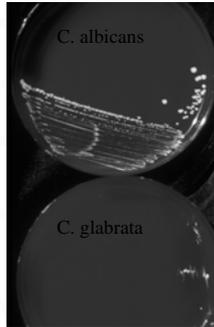
- Always start with a wet mount to confirm presence of yeast
- Cannot separate from *C. dubliniensis* which does not grow at 42°C-Not usually necessary
- *C. tropicalis* can form fringe (not feet) or get projections in germ tube after 3 h.

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Candida glabrata

- Colonies smaller on BAP than other yeast
- Yeast in wet mount are tiny with no hyphae
- Colonies are larger on EMB than BAP at 24 h or....

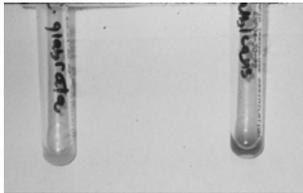
Represents 20% of yeast in urine



Growth in 24 h at 35°C

Candida glabrata

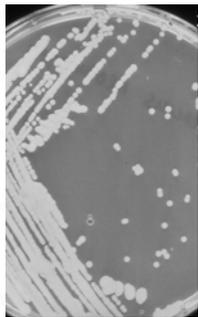
- Colonies smaller on BAP than other yeast
- Yeast in wet mount are tiny
- Positive (yellow) RAT test in 3 h at 42°C



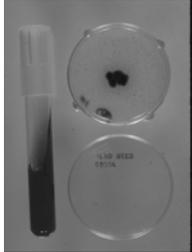
80

Cryptococcus neoformans

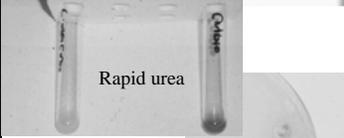
- Large mucoid colonies
- No pigment
- Capsule by India Ink or no pseudohyphae
- Round cells
- And positive caffeic acid test



Cryptococcus neoformans



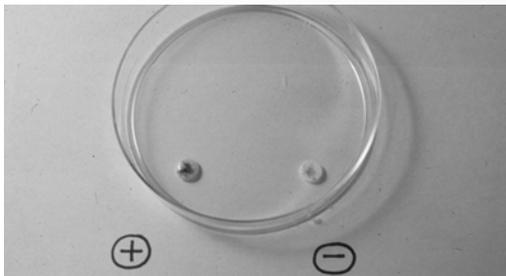
Overnight



Rapid urea

- Rapid caffeic acid
- Use media without dextrose
- 30°C 4 h is best

L-DOPA disk works better



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Rapid testing can make a difference

Thank you

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